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Effects of Chitosan Treatment on the Quality Parameters of Shrimp (*Parapenaeus longirostris*) during Chilled Storage

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Abstract

This study was conducted to investigate the effects of chitosan treatment on the quality criteria as of shrimps for a long time "in chilled" storage. Fresh deepwater pink shrimps (*Parapenaeus longirostris*), caught from Marmara Sea were dipped in different solutions containing the combinations of sodium metabisulphite (2500 mg/L), 4-hexylresorcinol (50 mg/L), chitosan (5 g/L), citric acid (200 mg/L) and rosemary extract (50 mg/L) for ten minute, over-wrapped in styrofoam plates and stored at 4°C for six days. During the storage period, pH, microbiological counts of total aerobic mesophilic bacteria (TAMB), total psychrotrophic bacteria (TPB), lactic acid bacteria (LAB), *Pseudomonas* spp. and Enterobacteriaceae, instrumental texture (shear force), moisture, TVB-N, TMA-N and sensory characteristics of shrimps were evaluated. TVB-N and TMA-N amounts in shrimps treated with polyphenol oxidase inhibitors alone are significantly higher than treated with chitosan throughout storage. Reduction in odor and flavour scores of chitosan treated samples was more slowly than others, while rosemary extract addition caused an undesirable flavor changes in shrimps. Coating with chitosan reduced moisture loss, textural softening and increase in pH of shrimps. The microbial counts of shrimps were gradually increase during storage, but addition of chitosan reduced the growth of spoilage bacteria and showed further inhibitory effect than samples treated with only antimelanosis agents. Incorporating of citric acid and rosemary extract into dipping solutions containing chitosan were inadequate to meet the commercial expectation. As a result, shrimps coated with chitosan after capture can provide an increase of up to twice the shelf-life by slowing down the biochemical reactions during cold storage.

Keywords:

Introduction

Shrimps are very rich in protein and free amino acids and poor in connective tissue. Moreover, they have a high a_w and a pH between 6.5-7.0. This means that shrimp is an ideal substrate for microbial growth. In addition, a rapid autolysis happens in shrimp tissue during post-mortem storage. Therefore, shrimps are highly perishable and have a short shelf-life (Shamshad *et al.*, 1990; Abu-Bakar *et al.*, 2008). In practice, shrimps are processed with polyphenol oxidase (PPO) inhibitors such as metabisulphite and 4-hexylresorcinol, drained and then packed with ice in order to maintain the quality (Mendes, 2006). Cold storage provides a limited increase for shelf-life of shrimp. During storage, protein, glycogen and fat content of shrimps are exposed to oxidation, hydrolysis and decomposition. The process results with formation of chemical metabolites such as TVB-N, TMA-N that can be used as deterioration indicator. Microorganisms which cause spoilage have significant role in formation of these metabolites. Commonly used melanosis inhibitors have antimicrobial effects, but their effects are not sufficient to increase the shelf-life (Nirmal and Benjakul, 2010). Stopping or slowing down the growth of existing microflora is essential to extend the shelf-life.

Chitosan, the deacetylated derivative of chitin, is a natural D-glucosamine polymer that can be extracted from the shells of seafood (Shahidi *et al.*, 1999; Rinaudo, 2006). It is biologically safe, non-toxic, biocompatible and biodegradable polysaccharide (Terbojevich and Muzarelli, 2000). Chitosan has been approved by FDA for use in certain food applications (FDA, 2005). Several studies have established that chitosan, as a natural preservative, is effective against a range of microorganisms (Liu *et al.*, 2001; Chung and Chen, 2008). Although there are several theories about the mechanism of the antimicrobial activity of chitosan, the common opinion is that chitosan disrupts the structure of the cellular membrane (Andres *et al.*, 2007; Helander *et al.*, 2001). Liu *et al.* (2004) reported that chitosan increased the permeability of the outer and inner membrane of *Escherichia coli* and *Staphylococcus aureus* and ultimately disrupted bacterial cell membranes, with the release of cellular contents, and

asserted that this damage was likely caused by the electrostatic interaction between NH_3^+ groups of chitosan acetate and phosphoryl groups of phospholipid components of cell membranes.

Several studies reported that chitosan extended the shelf-life of foodstuffs (Aldemir and Bostan, 2009; Mohan *et al.*, 2012). However, only a limited number of the mare present on the effect of chitosan derived from their shells on the quality parameters during storage of shrimps. From this point, this study was conducted to investigate the effect of chitosan treatment on the sensory and quality parameters of shrimp in combination with PPO inhibitors and other additives (citric acid, rosemary extract) during chilled storage.

Material and Methods

Shrimp Samples

The deepwater pink shrimps (*Parapenaeus longirostris*) were caught using a drag net (beam trawl) in Marmara Sea (Tekirdağ offshore). The shrimps were immediately mixed with ice in 2:1 ratio in polystyrene boxes after being caught, and were brought to the processing laboratory within 90 minutes. The shrimping was carried out on three different dates between May-June 2011 and approximately 25 kg of shelled shrimps were used in each experiment.

Treatment of Shrimps with Preservatives

Shrimps were washed with tap water in a large plastic bowl and were divided into eight equal batches of 3 kg each and treated for 10 min by dipping in 6 L of solutions prepared with sodium metabisulphite (Merck, 106528), 4-hexylresorcinol (Merck, 820647), deacetylated (75-85%) chitosan (Sigma-Aldrich 448877), acetic acid (Merck, 100056), citric acid (Merck 100244) and rosemary extract (Sigma-Aldrich, W299200) combinations at the ratios given on Table 1. (Preliminary analysis showed that the shrimps treated chitosan alone exposed to the discoloration in a very short period. Therefore, PPO inhibitors were included in all combinations). The active ingredients of Groups A and B were directly dissolved in tap water. For the other groups using

Table 1. Experimental design and composition of dipping solutions

Group	Acetic acid	Chitosan	Rosemary extract	Citric acid	Sodium metabisulphite	4-Hexylresorcinol
A	-	-	-	-	2500 mg/L	-
B	-	-	-	-	-	50 mg/L
C	10 ml/L	5 g/L	-	-	2500 mg/L	-
D	10 ml/L	5 g/L	-	-	-	50 mg/L
E	10 ml/L	5 g/L	50 mg/L	200 mg/L	2500 mg/L	-
F	10 ml/L	5 g/L	50 mg/L	200 mg/L	-	50 mg/L
G	10 ml/L	5 g/L	-	-	-	-

chitosan (C, D, E, F), first of all, 1% of acetic acid solution was prepared, then chemicals were added when chitosan was completely dissolved in this solution. After treatment, shrimps removed and drained for 10 minutes by using a filter (colander), and then each shrimp group was placed in styrofoam plates and covered with polyethylene film. Packages were kept in refrigerator (4°C) for 6 days and each group was periodically analyzed in terms of sensorial and chemical qualities at 2, 4, and 6 days of chilled storage.

Microbiological Analysis

Twenty five grams of chopped shrimps from each group at each storage interval was transferred to a sterile bag with 250 mL sterile peptone water (Oxoid, CM0009) and was homogenized for 90 sec using a stomacher (LabBlender 400, Steward Lab., UK). Serial decimal dilutions were prepared using the same diluents. A 0.1- or 1-mL inoculum of appropriate dilutions was plated on Standard Plate Count agar (Merck 1.05463), and poured plates were incubated at 35°C for 48 hours for total aerobic mesophilic bacteria (TAMB) counts, and at 7°C for 10 days for total psychrotrophic bacteria (TPB) counts (Harrigan, 1998). Lactic acid bacteria (LAB) was enumerated on de Man, Rogosa, Sharpe agar (Merck, 1.10660) by incubating poured plates at 30°C for 72 hours (ISO, 1998). *Pseudomonas* spp. were determined on Pseudomonas agar (Merck, 1.07620) supplemented with *Pseudomonas* CFC selective supplement (Merck, 1.07627) after incubation at 25°C for 48 hours and confirmed by the oxidase test (Merck, 1.13300) (ISO, 2000). *Enterobacteriaceae* were examined in Modified HiCrome UTI Agar (Himedia, M1418) by incubating spread plates at 25–30°C for 24 h and enumerating all blue and red colonies (Merlino et al., 1996). Results are recorded as the arithmetic means of the duplicate tests at three independent time and were expressed as log₁₀ cfu/g.

Determination of pH

Ten gram of shrimps were homogenized and mixed thoroughly with 100 mL of distilled water for measuring of pH using a digital pH meter (Hanna HI 9125) at room temperature (AOAC, 1990).

Determination of Moisture Content

Approximately 5 g of samples were weighed and dried in an oven (Heraeus, Germany) at 115°C more than three hours until a constant weight was determined (AOAC, 1990).

Determination of Trimethylamin Nitrogen (TMA-N)

Ten grams of homogenized samples were weighed, blended with 90 mL of 7.5% trichloroacetic acid solution and filtrated. Four milliliters extract was transferred into test tubes and 1 mL of formaldehyde, 10 mL of anhydrous toluene, and 3 mL of K₂CO₃ solution were added. The tubes were shaken and 5 mL of toluene layer was pipetted. Five millilitres of picric acid solution (0.02%) was added. The contents were mixed and transferred to a spectrophotometric cell and absorbance was measured at 410 nm against the blank. At the same time, standards were prepared and measured (AOAC, 1998). Results of TMA-N were expressed as mg/100 g sample.

Determination of Total Volatile Basic Nitrogen (TVB-N)

The vapour distillation method (Manthey and Oehlenschlaeger, 1983) was used to determine the amount of TVB-N. Samples were boiled with catalyst (MgO), and vapour components were trapped with hydrochloric acid (0.1 N). The amount of TVB-N was calculated after titration with sodium hydroxide (0.1 N). Results of TVB-N were expressed as mg/100 g sample.

Texture (Shear Force) Analysis

Shear force was measured using a texture analyzer equipped with a Warner-Bratzler shear device (3343 model, Instron, UK). Raw shrimp samples without shell were placed on the flat base plate of measuring machine and each measurement was made from the second segment of shrimps. A graph of high strength (kg/cm²) and force x time was obtained using 50 mm/min of constant speed and cutting up to 70 % of the deformation depth of the samples. The results of each group were evaluated after calculating the arithmetic means of 15 different shrimps (Niamnuy et al., 2007).

Sensorial Evaluation

Raw and cooked (kept in boiling water for 3 minutes, cooled and peeled) shrimps of each group at each storage interval were presented to eight trained panellists (2 females and 6 males), ranging in ages between 26 and 45 years and trained according to ISO (1993). Prior to the analysis, vocabularies of the sensory attribute were developed by the panellists in a round-table session, using a standardized procedure (ISO, 1998), and training was conducted in two separate sessions approximately 2 h for the evaluation of selected attributes and was followed by an open-discussion session to familiarize panellists with the attributes and the scale to be used. All assessments took place in individual temperature-controlled booths in a day-light conditions. Each sample was labelled, at random, with a three-digit code number. The panelists

were received a set of 6 samples (with 3 samples of in each plate) served in a complete randomized order. It was requested to evaluate odour, texture (firmness), flavor (for only cooked samples) and overall acceptability of shrimps by marking a position on the unstructured line scale (a graphic rating scale having a 100 mm line, 0: unacceptable; 100: perfect). Ratings were measured by a ruler and converted to numerical scores and the means of panellists scores were defined using SPSS programs (Botta, 1995).

Statistical Analysis

Analysis of variance (ANOVA) was conducted for each variable measured to investigate the effect of treatments during storage time. The trial was performed triplicate and significant differences were defined with SPSS 13.0 using Tukey's and Duncan's multiple range tests ($P < 0.05$) (SPSS, 2001).

Results and Discussion

The changes in TAMB and TPB counts during refrigerated storage are summarized in Table 2. Before treatment, the initial TAMB and TPB of shrimps were 4.56 and 3.83 log cfu/g, respectively and reduced up to 0.3-0.9 log for mesophilic bacteria and 0.9-1.5 log for psychrotrophic bacteria depending on the chemical treatments which proves that the melanosis inhibitor has also antibacterial effect. In fact, the combination of chitosan with melanosis inhibitors resulted in higher reduction. The total bacteria counts in all groups gradually increased during storage, while the counts in samples treated with chitosan containing solutions were lower than samples treated with only sodium metabisulphite or 4-hexylresorcinol at each stage of storage. Likewise, Mohan *et al.* (2012) reported that chitosan treatment lowered the total bacterial counts in sardines with an increase more slowly than others during storage. Tsai *et al.* (2002) also emphasized that addition of 1% chitosan solution retarded the increase in the counts of mesophilic and psychrotrophic bacteria in fish fillets. In the present study, the highest antimicrobial effects was found in the chitosan groups' fortified with the rosemary extract and citric acid, but this effect between groups has not significant difference ($P > 0.05$).

Bacteriological spoilage in refrigerated fish and fish products under aerobic storage conditions is caused by gram-negative psychrotrophic organisms such as *Pseudomonas* spp. (Hubbs, 1991). Studies on the bacteriological quality of shrimp reported that *Pseudomonas* was the dominant bacterial flora during refrigerated storage (Jeyasekaran *et al.*, 2006; Lalitha and Surendran, 2006). In the present study, the mean count of *Pseudomonas*, which is 2.06 log cfu/g on the untreated samples, was determined between 1.43-1.79 log cfu/g after treatment, but no significant difference was observed among groups ($P > 0.05$). *Pseudomonas* counts increased gradually with storage time (Table 2)

and may play an important role in the deterioration of refrigerated shrimps besides the effect of other microflora and autolytic factors in the decay process. Meanwhile, chitosan treatment reduced the growth of *Pseudomonas* in shrimps reaching 4.43-5.48 log cfu/g at the end of the storage, whereas the counts for those treated with only melanosis inhibitors were about 6.5 log cfu/g. Additionally, the inhibitory effect of chitosan was significantly enhanced with citric acid and rosemary extract supplementation. Similarly, Chen *et al.* (1998) reported that the growth of *Pseudomonas* on oysters was retarded by chitosan addition.

The initial enteric bacterial counts were determined to be very close to the total bacteria counts (Table 2) and a fast increase was recorded in enteric bacterial counts during cold storage. Similar results were reported in previous studies (Mendes *et al.*, 2002; Nirmal and Benjakul, 2012). It was observed that the majority of *Enterobacteriaceae* counts in the present study was consisted of *Enterococcus faecalis*. On the basis of this results, it can be concluded that *E. faecalis* would be among the bacteria facilitating the decay of shrimps. Dipping into the solutions with chitosan significantly reduced the *Enterobacteriaceae* counts of shrimps, and inhibited their growth during storage (Table 2). Likewise, Chen *et al.* (1998), Tsai *et al.* (2002) and Georgantelis *et al.* (2007) indicated that the inhibitory effect enhanced with chitosan treatments.

Lactic acid bacteria are among the microorganisms which cause spoilage for most foodstuffs. Jeyasekaran *et al.* (2006) stated that LAB counts of shrimps kept in ice were determined as 10^2 - 10^6 cfu/g. In the present study, LAB counts of shrimp were not detectable level (< 1.0 log cfu/g) in the beginning of the storage; but increased slowly (2.30-2.91 log cfu/g at the 6th day) in all groups during storage period (Table 2). However, their levels were not sufficient to cause microbial deterioration in shrimps, it can be interpreted that LAB is not an important part of spoilage flora in some cases for shrimps.

During storage of shrimps, the increase in the pH value is associated with the accumulation of basic compounds as a result of the enzymatic activity, both endogenous and microbial (Lopez-Caballero *et al.*, 2007). In the present study, pH value of fresh shrimps was determined between 6.67-6.82. Initial pH value increased rapidly and steadily during storage and reached to 7.65 and 8.23 on the 6th day (Table 3). Similarly to our findings, Varlık *et al.* (2000) determined that pH value of shrimps stored in refrigerator was increased from 6.73 to 7.81 at the 4th day of storage. Bilgin *et al.* (2006) reported that pH value of brown shrimps kept in refrigerator was 6.83 and increased up to 7.95 on the 5th day. Erdem and Bilgin (2004) reported that pH value of raw shrimps (*Palaemonads persus*) was 7.60 at the beginning of cold storage period and increased up to 8.06 on the 5th

Table 2. Mean counts of bacteria in shrimps during chilled storage (4°C) (log cfu/g)

Grup	Chilled storage (day)				
	0	2	4	6	
TAMB	A	4.11±0.14 ^{ab}	5.33±0.11 ^b	6.88±0.13 ^a	7.64±0.12 ^a
	B	4.28±0.17 ^a	5.67±0.11 ^a	6.68±0.10 ^{ab}	7.44±0.12 ^{ab}
	C	3.72±0.11 ^{bc}	4.55±0.09 ^c	6.28±0.13 ^{bc}	7.18±0.14 ^b
	D	3.64±0.13 ^c	4.61±0.14 ^{bc}	6.13±0.13 ^c	7.04±0.11 ^b
	E	3.75±0.13 ^{bc}	4.89±0.11 ^{bc}	6.30±0.20 ^{bc}	7.09±0.13 ^b
	F	3.86±0.09 ^{bc}	4.92±0.11 ^b	6.42±0.16 ^{bc}	7.16±0.12 ^b
	P	*	***	*	*
TPB	A	2.84±0.09 ^{ab}	4.69±0.12 ^{ab}	5.90±0.12 ^{ab}	7.16±0.14 ^{ab}
	B	2.92±0.11 ^a	4.97±0.15 ^a	6.16±0.12 ^a	7.38±0.14 ^a
	C	2.54±0.13 ^{bc}	4.42±0.12 ^{bc}	5.65±0.13 ^{bc}	6.78±0.16 ^b
	D	2.49±0.08 ^c	4.35±0.18 ^{bc}	5.42±0.17 ^c	6.85±0.10 ^b
	E	2.33±0.11 ^c	4.14±0.12 ^c	5.45±0.11 ^c	6.98±0.10 ^{ab}
	F	2.37±0.11 ^c	4.20±0.13 ^c	5.60±0.12 ^{bc}	7.12±0.16 ^{ab}
	P	*	**	*	*
Pseudomonas spp.	A	1.70±0.12	3.32±0.13 ^{ab}	4.73±0.15 ^a	6.48±0.14 ^a
	B	1.79±0.12	3.52±0.14 ^a	4.74±0.11 ^a	6.61±0.13 ^a
	C	1.64±0.08	3.02±0.14 ^b	4.59±0.13 ^a	5.48±0.12 ^b
	D	1.60±0.12	2.89±0.14 ^{bc}	4.41±0.15 ^a	5.03±0.12 ^c
	E	1.46±0.17	2.58±0.12 ^c	3.76±0.13 ^b	4.57±0.15 ^d
	F	1.43±0.16	2.56±0.14 ^c	3.56±0.18 ^b	4.43±0.13 ^d
	P	NS	***	***	***
Enterobacteriaceae	A	3.77±0.09 ^a	5.15±0.13 ^a	6.28±0.15 ^a	7.11±0.14 ^a
	B	3.55±0.16 ^a	4.96±0.11 ^a	6.14±0.18 ^{ab}	6.88±0.17 ^a
	C	2.91±0.15 ^b	4.40±0.13 ^b	5.87±0.17 ^{abc}	6.38±0.15 ^b
	D	2.94±0.14 ^b	4.22±0.16 ^{bc}	5.74±0.13 ^{bc}	6.21±0.08 ^b
	E	2.67±0.15 ^b	3.85±0.12 ^c	5.52±0.09 ^c	6.04±0.16 ^b
	F	2.58±0.17 ^b	3.81±0.13 ^c	5.42±0.14 ^c	6.09±0.13 ^b
	P	***	***	**	***
LAB	A	<1.0	1.49±0.08 ^a	1.80±0.12 ^c	2.38±0.08 ^{cd}
	B	<1.0	1.06±0.16 ^b	1.69±0.08 ^c	2.30±0.06 ^d
	C	<1.0	1.85±0.21 ^a	2.26±0.10 ^b	2.67±0.02 ^{ab}
	D	<1.0	1.69±0.09 ^a	2.25±0.08 ^b	2.61±0.11 ^{bc}
	E	<1.0	1.96±0.11 ^a	2.58±0.10 ^a	2.91±0.13 ^a
	F	<1.0	1.55±0.17 ^a	2.17±0.12 ^b	2.62±0.09 ^{bc}
	P	NS	*	***	**

^{a,b} Means within a column with different letters are significantly different (P<0.05). (*): P<0.05, (**): P<0.01, (***): P<0.001, (NS): not significant.

(A) sodium metabisulphite (2500 mg/L), (B) 4-hexylresorcinol (50 mg/L), (C) chitosan (5 g/L) + sodium metabisulphite (2500 mg/L), (D) chitosan (5 g/L) + 4-hexylresorcinol (50 mg/L), (E) chitosan (5 g/L) + citric acid (200 mg/L) + rosemary extract (50 mg/L) + sodium metabisulphite (2500 mg/L), (F) chitosan (5 g/L) + citric acid (200 mg/L) + rosemary extract (50 mg/L) + 4-hexylresorcinol (50 mg/L).

day. Other studies displayed a relatively slow increase of pH values during cold storage (Abu-Bakar *et al.*, 2008; Nirmal and Benjakul, 2010; Mendes *et al.*, 2002; Nirmal and Benjakul, 2009). The differences between studies in terms of increase in pH values can be associated with microbial loads of shrimps. Generally, shrimps with a pH of 7.5 and above are described as deteriorated product (Shamshad *et al.*, 1990; Abu-Bakar *et al.*, 2008).

Treatment with chitosan was in capable to significantly slow down the increase in pH value of shrimps. Mohan *et al.* (2012) investigated the effectiveness of chitosan coating on pH changes in sardine, and reported that untreated sardine showed a faster increase during storage period, while chitosan treated samples was significantly (P<0.05) lower pH

value than the untreated ones.

Dehydration is considered to be an important loss of quality during storage of most foodstuffs. In the present study, the moisture rates of samples partially decreased during storage. Chitosan dipping reduced the loss of moisture. The rates of moisture of chitosan treated samples were significantly higher than others (Table 3). The moisture loss is due to dehydration by evaporating or leaking of the existing water from shrimp tissue. The current findings suggest that covering shrimp with chitosan prevents dehydration in vapor phase and thus effectively decreases the loss of moisture. On the other hand, some authors stated that although chitosan film was highly impermeable to oxygen, it had poor water vapour barrier property (Butler *et al.*, 1996; Xu *et al.*, 2005). Yingyuad *et al.*

Table 3. Physical and chemical quality changes in shrimps during chilled storage (4°C)

Group	Chilled storage (day)				
	0	2	4	6	
pH	A	6.82±0.09	7.29±0.06 ^{ab}	7.74±0.09 ^a	8.23±0.11 ^a
	B	6.81±0.06	7.35±0.06 ^a	7.80±0.08 ^a	8.12±0.11 ^a
	C	6.74±0.07	7.13±0.07 ^{ab}	7.45±0.11 ^{ab}	7.77±0.08 ^{ab}
	D	6.82±0.05	7.12±0.06 ^{ab}	7.39±0.07 ^{ab}	7.69±0.08 ^{ab}
	E	6.67±0.05	7.05±0.05 ^{ab}	7.45±0.07 ^{ab}	7.71±0.07 ^{ab}
	F	6.67±0.06	6.98±0.08 ^{ab}	7.32±0.07 ^{ab}	7.65±0.10 ^{ab}
	P	NS	**	**	***
Moisture (%)	A	77.89±0.40	77.45±0.37	77.03±0.31 ^{ab}	76.43±0.22 ^b
	B	77.68±0.13	77.30±0.18	76.78±0.21 ^b	76.39±0.21 ^b
	C	77.95±0.18	77.78±0.15	77.56±0.13 ^a	77.14±0.17 ^a
	D	77.88±0.17	77.76±0.11	77.49±0.11 ^a	77.21±0.12 ^a
	E	77.72±0.16	77.63±0.10	77.45±0.11 ^a	77.07±0.14 ^a
	F	77.80±0.21	77.67±0.26	77.42±0.15 ^a	77.13±0.18 ^a
	P	NS	NS	*	**
TMA-N (mg/100g)	A	0.24±0.03	8.10±0.51 ^a	23.56±2.25 ^a	35.62±2.37 ^a
	B	0.30±0.06	7.64±0.67 ^a	25.30±1.86 ^a	36.38±1.45 ^a
	C	0.28±0.06	4.43±0.50 ^b	15.77±0.91 ^b	26.80±1.25 ^{bc}
	D	0.27±0.04	4.06±0.49 ^b	16.84±1.43 ^b	29.44±1.07 ^b
	E	0.33±0.04	3.79±0.56 ^b	14.64±1.16 ^b	23.63±2.06 ^c
	F	0.26±0.03	4.13±0.45 ^b	15.41±1.30 ^b	25.33±1.58 ^{bc}
	P	NS	***	***	***
TVB-N (mg/100g)	A	13.35±1.51	33.50±2.01 ^a	71.87±3.22 ^a	140.56±3.78 ^a
	B	13.79±1.79	35.92±1.92 ^a	73.49±2.31 ^a	146.62±3.54 ^a
	C	13.50±1.08	24.67±2.58 ^b	48.01±2.76 ^b	112.29±3.58 ^b
	D	13.06±1.24	25.19±2.73 ^b	50.19±1.85 ^b	116.13±3.73 ^b
	E	13.66±1.07	21.29±2.23 ^b	42.26±2.16 ^b	105.35±3.24 ^b
	F	13.45±1.32	21.69±2.45 ^b	47.18±2.71 ^b	108.24±2.47 ^b
	P	NS	**	***	***
Shear force (kg/cm ²)	A	0.92±0.03	1.13±0.03	0.82±0.03 ^c	0.51±0.04 ^c
	B	0.89±0.04	1.14±0.02	0.90±0.03 ^{bc}	0.60±0.04 ^c
	C	0.93±0.02	1.21±0.02	1.02±0.05 ^{ab}	0.78±0.04 ^{ab}
	D	0.88±0.03	1.19±0.02	1.05±0.05 ^a	0.75±0.05 ^b
	E	0.90±0.02	1.16±0.04	1.14±0.06 ^a	0.88±0.04 ^a
	F	0.92±0.03	1.20±0.02	1.10±0.04 ^a	0.83±0.03 ^{ab}
	P	NS	NS	***	***

^{a,b}Means within a column with different letters are significantly different (P<0.05). (*): P<0.05, (**): P<0.01, (***): P<0.001, (NS): not significant.

(A) sodium metabisulphite (2500 mg/L), (B) 4-hexylresorcinol (50 mg/L), (C) chitosan (5 g/L) + sodium metabisulphite (2500 mg/L), (D) chitosan (5 g/L) + 4-hexylresorcinol (50 mg/L), (E) chitosan (5 g/L) + citric acid (200 mg/L) + rosemary extract (50 mg/L) + sodium metabisulphite (2500 mg/L), (F) chitosan (5 g/L) + citric acid (200 mg/L) + rosemary extract (50 mg/L) + 4-hexylresorcinol (50 mg/L).

(2006) reported that chitosan coating did not protect the grilled porks against moisture loss probably because of large volume of NH₂ side groups on chitosan which has strong affinity to water molecules. In our study, shell of shrimps may have been effective on the moisture loss in combination with chitosan. In addition, chitosan may have indirectly contributed by preventing the leakage resulted from tissue softening due to its retarding effect of the microbial deterioration.

In the present study, pH, TMA-N and TVB-N values obtained during storage of shrimps also confirmed that chitosan delayed deterioration process. Amount of TMA-N is a substantial indicator of freshness for fish and shellfish. There is also wide variation in critical values suggested for individual species. This value was recommended as 8 mg/100 g

for shrimps (Varlık *et al.*, 2000; Malle and Poumeyrol, 1989). In the present study, the initial TMA-N contents of shrimps were determined between 0.24-0.33 mg/100g. The value rapidly increased during storage and exceeded the stated limits on the 4th day in all groups (Table 3). Other studies conducted with shrimps stated that amount of TMA-N exceeded the level of 8 mg/100 g within 4 to 8 days (Varlık *et al.*, 2000; Bilgin *et al.*, 2006; Erdem and Bilgin, 2004; Stockemer and Nieper, 1984). The TMA-N content of shrimps processed with chitosan increased significantly slower in comparison to the shrimps not processed with chitosan during refrigerated storage. This can be explained with antimicrobial effect of chitosan. Padio *et al.* (2011) argued that there is a direct relationship between TMA-N level of shrimps and the count of

psychrotrophic bacteria.

TVB-N, which is useful indices of spoilage in different fresh and lightly preserved seafood, includes primarily trimethylamine, ammonia, and dimethylamine (Malle and Poumeyrol, 1989). Critical limits of (25-40 mg-TVBN/100 g) were established for different groups of sea foods. Limits of TVB-N of 30 mg N/100 g in shrimp have been reported (Cobb et al., 1976). In the present study, the rapid increase of TVB-N content of shrimps during storage was observed (Table 3). In some groups, level of 30 mg/100 g was exceeded on the second day of storage, and in all groups, the amounts of TVB-N were above the upper limit on the 4th day. A rapid TVB-N (increase??) in untreated shrimp samples was reported in a short time in most of the even though they were stored in refrigeration temperatures as in our study. Erdem and Bilgin (2004) reported that TVB-N content of raw shrimp (*Palaemonadispersus*) stored in refrigerator (4°C) was increased to the level of 44.64 mg/100 g on the 5th day of storage, whereas it was 1.02 mg/100 g at the beginning. Stockemer and Nieper (1984) reported that TVB-N content of brown shrimps (*Crangoncrangon*) stored at 7°C was 37.1 mg/100 g on the 4th day and 150 mg/100 g on the 6th day. Varlık et al. (2000) emphasized that initial TVB-N value of shrimps stored in cold chain (4°C) was 22.9 mg/100 g and increased up to 109.15 mg/100 g on the 4th day of storage. Bilgin et al. (2006) reported that the amount of TVB-N in the brown shrimps stored in refrigerator was reached to 42.53 mg/100 g on the 5th day, whereas this value was 8.87 mg/100 g at the beginning. In some other studies, slower increase for TVB-N content was determined during storage. Shams had et al. (1990) found an increase in TVB-N values of untreated shrimps from 4.5 to 16.7 mg/100 g after 9 days of storage at 5°C. However, Abu-Bakar et al. (2008) observed that TVB-N content in freshwater prawn sample in iced storage exceeded the level of 30 mg/100 g at the 16th day. Cheuk et al. (1979) reported that the upper limit in iced storage brown and rose shrimps was reached after 12-16 days of storage. TVB-N level in shrimps is closely related to the microbial quality, as well as other factors (Malle and Poumeyrol, 1989). The differences between studies may be due to the different microbial loads of shrimps.

TVB-N content of shrimps treated with chitosan was significantly lower than shrimps treated with common PPO inhibitors at all stages of storage (Table 3). The results indicated that chitosan treatment inhibited the formation of TVB-N. Mohan et al. (2012) examined the effectiveness of edible chitosan coating on TVB-N level in sardines, and reported that, on the day of sensory rejection for untreated samples, the formation of TVB-N was less by 14.9% for 1% chitosan treated sardine and 32.7% for 2% chitosan treated samples. Muşabak (2008) who studied the effects of chitosan (2%) coating on chemical parameters of Atlantic bonito (*Sardasarda*)

fillets determined that TVB-N values were significantly lower in chitosan group than control group during storage. In another study, Tsai et al. (2002) found that coating with chitosan significantly delayed the increase of TVB-N content on fish fillets during storage.

The sensorial assessments showed that texture (firmness) scores of all groups gradually decreased during storage indicating to softening (Figure 1). Shrimps become loose and juicy in a varying period depending on storage temperature after catching. Softening of texture originates from biochemical activities including bacterial and autolytic enzymes. On the other hand, muscle proteins become watery due to the increased water binding capacity as a result of the increased pH value during storage, and thus muscle tissue becomes softer (Ray, 2004). In the present study, rapid increase in pH value is also parallel to the tissue softening. The shear force of shrimps partially increased on the 2nd day of storage but then displayed a tendency to decrease (Table 3). The slight increase of shear force in the first days can be explained by probably water leaking from tissue to harden it. Subsequent decreasing may be due to excessively decomposition of tissue components.

Although the texture points of shrimps treated with chitosan were relatively higher, there was no statistically significant difference between groups ($P > 0.005$). However, chitosan treated samples had significantly higher shear force values than the ones treated with both sodium metabisulphite and 4-hexylresorcinol on the 6th day of storage. It is known that chitosan improves tissue characteristics. But, the difference in this study may be associated with earlier deterioration of shrimps treated only PPO inhibitors. Contrary results have been obtained in other studies conducted in different foods. Mohan et al. (2012) stated that chitosan coating sardine fillets improved the textural properties significantly compared to untreated ones. Yingyuad et al. (2006) determined no significant texture differences among packaged grilled pork with and without chitosan coating during the whole storage period.

Changes in colour during storage were defined sensorially and instrumentally in previously published article (Varlık et al., 2014). It was well demonstrated that chitosan alone had no effect on discoloration, but in combination with PPO inhibitors showed an additional inhibitory effect on melanosis formation. All groups scored less than half of maximum score on the 4th day to the panellists and it was concluded that 50 mg/L of 4-hexylresorcinol were more effective than 2500 mg/L of sodium metabisulphite in the prevention of melanosis development after shrimping. Paralelly to the sensory scores, lightness (L^*) value that is directly associated with melanosis, decrease continuously on the storage period and a similar decrease was observed for redness (a^*) values. However, yellowness (b^*) value, which is associated with deterioration was increased in relation to the

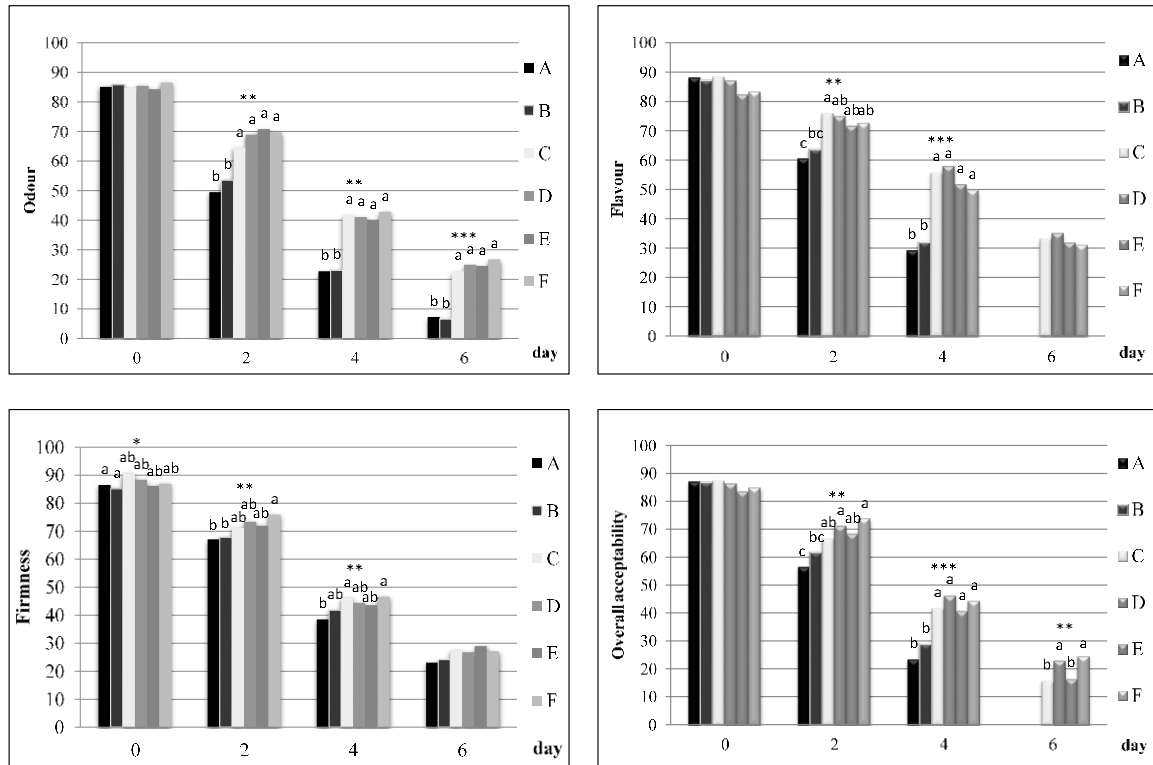


Figure 1. Changes in sensory scores of shrimp during the chilled storage period (on the sixth day, overall acceptability and flavour evaluation for the A and B groups were not conducted due to excessive decomposition).

(A) sodium metabisulphite (2500 mg/L), (B) 4-hexylresorcinol (50 mg/L), (C) chitosan (5 g/L) + sodium metabisulphite (2500 mg/L), (D) chitosan (5 g/L) + 4-hexylresorcinol (50 mg/L), (E) chitosan (5 g/L) + citric acid (200 mg/L) + rosemary extract (50 mg/L) + sodium metabisulphite (2500 mg/L), (F) chitosan (5 g/L) + citric acid (200 mg/L) + rosemary extract (50 mg/L) + 4-hexylresorcinol (50 mg/L).

^{a,b}Means within a column with different letters are significantly different (P<0.05). (*): P<0.05, (**): P<0.01, (***): P<0.001, (NS): not significant.

microbial growth in all groups.

Regardless of the beneficial effects, food additives may cause adverse changes in foods. In the present study, sensory characteristics of shrimps were not adversely affected by different treatment combinations. Odour and flavour changes that affect the acceptability of shrimps occurs in a period of time ranging depending on the quality and storage conditions of raw material. Bilgin *et al.* (2006) reported that the initial odour and flavour scores of brown shrimps (*Crangon crangon*) kept in the refrigerator were determined as 8.70 and 8.82, respectively, however these scores were decreased to 1.49 and 1.87 on the 5th day of storage. Erdem and Bilgin (2004) reported that the odour and flavour scores in the shrimp (*Palaemon adspersus*) stored at refrigerated temperatures (4°C) reduced from 8.71 and 8.71 at the beginning to 3.08 and 3.17 at the 3rd day of storage, respectively. Varlık *et al.* (2000) determined that the cold stored shrimps have become sensorially unacceptable in four days. Stockemer and Nieper (1984) found that the untreated shrimps stored at 7°C became unacceptable on the 4th day of storage. In the present study, shrimps were graded with scores indicating spoilage in four and six days of chilled storage similarly to the results of the above-mentioned studies. On the other hand, there are

studies indicating a longer shelf-life during cold storage. For examples, Pardio *et al.* (2011), who studied the sensory changes in shrimp (*Panaeus aztecus*) during at -1°C, reported that the untreated control samples were scored as putrid flavour at day 19 of storage. This difference in shelf-life related to lower storage temperature as well as other factors.

The panelists gave a mean odour score of approximately 85 over 100 point for shrimps in the beginning, but the scores rapidly decreased during refrigerated storage (Figure 1). In evaluation at the 4th day, Group A and B shrimps treated with only sodium metabisulphite and 4-hexylresorcinol received about 20 point, whereas the chitosan treated samples (C, D, E and F group) had significantly higher scores (between 40.0 and 42.9). Similar results were obtained from flavour evaluation. Shrimps that scored almost a perfect point at the beginning were exposed to significantly decreased points during subsequent days of storage.

In Group A and B shrimps processed with only common PPO inhibitors, flavour scores decreased more rapidly than in Group C-E shrimps processed with chitosan. The changes in odour and flavour points affected directly the overall acceptability points. Existing findings indicate that the application

of chitosan coating decreased the severity of sensorial changes of shrimps and markedly improved sensory quality. Other researches also confirmed that chitosan treatment delays the sensorial changes in different foodstuffs. Mohan *et al.* (2012) studied the effectiveness of edible chitosan coating on the quality changes of Indian oil sardine in iced condition, and concluded that the eating quality was maintained up to 8 and 10 days for 1 and 2% chitosan treated samples respectively, compared to only 5 days for untreated samples. Tsai *et al.* (2002) also observed that the shelf-life of salmon fillets dipped in chitosan solution (1%) was extended from 5 to 9 days. Yinyuad *et al.* (2006) investigated the effects of chitosan coating on the quality and shelf-life of retail packaged grilled pork during refrigerated storage, and reported that vacuum-packaged samples without chitosan coating reached unacceptable sensory scores after 14 days, while samples coated with chitosan maintained acceptable sensory qualities throughout the storage period up to 28 days. Chitosan, by inhibiting the microorganisms, retards spoilage and thus slows down the formation of degradation products that cause odour and flavour changes.

In the present study, the combinations of chitosan with citric acid and rosemary extract were included in the experiments to determine the possible synergistic effect. Although most of parameters were partially improved, there was no statistically significant difference between groups. Moreover, the addition of rosemary extract (50 mg/L) to the dipping solution negatively affected the flavour of shrimps. The panelists expressed the taste and smell of rosemary as a score lowering factor. Citric acid can create a synergistic effect with antimicrobials used in conjunction and increase their effectiveness (Theron and Lues, 2010). Rosemary extracts have a potent antioxidant activity and are widely used in food industry. In addition to inhibition of lipid oxidation, several authors have been reported that some of the compounds present in rosemary extracts inhibited or slowed the growth of some microorganism and thus delayed the process of spoilage (McBride *et al.*, 2007; Uçak *et al.*, 2011). In the present study, the unsatisfactory effect of rosemary extract or citric acid may be associated with the low application of low dose or very rapid progression of degradation process in shrimps.

The results of the present study pointed out that dipping into solutions containing chitosan enhanced the sensory acceptability period of shelled shrimps. This effect of chitosan has been associated with slowing down of the formation of TVB-N and TMA-N by its antimicrobial activity. In addition to positive effects of chitosan on quality criteria of shrimps, the reducing on the loss of moisture with chitosan is also considered to be an important advantage. Addition of rosemary extract and citric acid to the solutions containing chitosan was insufficient to meet the commercial expectations. It is known that consumers

have a negative approach against synthetic chemical preservatives. The availability of chitosan to enhance the shelf-life of foodstuffs as well as functional characteristics will provide a significant advantage in meeting the consumer demand as a natural alternative. However, dissolving of chitosan in only acidic solutions may cause difficulty in practical application. In case of the production of water-soluble chitosan preparations, application will become widespread. The use of chitosan in food industry will also contribute to recycling of large amounts of chitin separated as waste in shellfish processing.

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References

- Abu-Bakar, F., Salleh, A. B., Razak, C. N. A., Basri, M., Ching, M. K. and Son, R. 2008. Biochemical changes of fresh and preserved freshwater prawns (*Macrobrachium rosenbergii*) during storage. *International Food Research Journal*, 15: 181-191.
- Aldemir, T. and Bostan, K. 2009. Effect of chitosan on the microbiological quality of ready to cook meatball. *Journal of The Faculty of Veterinary Medicine, Istanbul University*, 35(2): 13-21.
- Andres, Y., Giraud, L., Gerente, C. and Le Cloirec, P., 2007. Antibacterial effects of chitosan powder: mechanisms of action. *Environmental Technology*, 28(12): 1357-1363. doi: 10.1080/09593332808618893
- AOAC, 1990. Association of Official Analytical Chemists. Official methods of analysis (Fifteen Edition). Arlington, VA.
- AOAC, 1998. Official Method 971.14: Trimethylamine nitrogen in seafood, In: Official Methods of Analysis of AOAC International, Ed. Cunniff, P., AOAC International, Chapter 35, p.7, Arlington, VA.
- Bilgin, S., Erdem, M. E. and Duyar, H. A., 2006. Chemical quality changes of Brown shrimp, Crangon Crangon (Linnaeus, 1758), stored at refrigerated temperatures as boiling and raw. *Firat University Journal of Science and Engineering*, 18(2): 171-179.
- Botta, J. R. 1995. Evaluation of seafood freshness quality. VCH Publishers. New York, USA, pp. 65-143.
- Butler, B. L., Vergano, P. J., Testin, R. F., Bunn, J. N. and Wiles, J. L. 1996. Mechanical and barrier properties of edible chitosan films as affected by composition and storage. *Journal of Food Science*, 61: 953-955. doi: 10.1111/j.1365-2621.1996.tb10909.x
- Chen C., Liao W. and Tsai G. 1998. Antibacterial effects of N-Sulfonated and N-Sulfobenzoyl chitosan and application to oyster preservation. *Journal of Food Protection*, 61: 1124-1128.
- Cheuk, W. L., Finne, G. and Nickelson, R. 1979. Stability of adenosine deaminase during ice storage of pink and brown shrimp from Gulf of Mexico. *Journal of Food Science*, 44:1625-1633. doi: 10.1111/j.1365-2621.1979.tb09103.x
- Chung, Y. C. and Chen, C. Y. 2008. Antibacterial

- characteristics and activity of acid-soluble chitosan. *Bioresource Technology*, 99: 2806-2814. doi:10.1016/j.biortech.2007.06.044
- Cobb, B. F., Vanderzant, C., Hanna, M. O. and Yeh, C. P. 1976. Effect of ice storage on microbiological and chemical changes in shrimp and melting ice in a model system. *Journal of Food Science*, 41: 29-34. doi: 10.1111/j.1365-2621.1976.tb01094.x
- Erdem, M. E. and Bilgin, S. 2004. Researches on quality changes of shrimp (*Palaemonadspersus Rathke*, 1837) stored at refrigerated temperatures as boiling and raw. *Firat University Journal of Science and Engineering*, 16(4): 687-694.
- Food and Drug Administration (FDA), 2005. Shrimp-Derived Chitosan GRAS Notification, http://www.accessdata.fda.gov/scripts/fcn/gras_notice/s/grn000170.pdf.
- Georgantelis D., Ambrosiadis I., Katikou P., Blekas G. and Georgakis S.A. 2007. Effect of rosemary extract, chitosan and α -tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4°C. *Meat Science*, 76: 172–181. doi:10.1016/j.meatsci.2006.10.026
- Harrigan, W. F. 1998. *Laboratory methods in food microbiology*. London: Academic Press.
- Helander, I. M., Nurmiäho-Lassila, E.-L., Ahvenainen, R., Rhoades, J. and Roller, S. 2001. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *International Journal of Food Microbiology*, 71(2-3): 235-244. doi:10.1016/S0168-1605(01)00609-2
- Hubbs J. 1991. Fish: microbiological spoilage and safety. *Food Science and Technology Today*, 5: 166–173.
- International Standardization Organization (ISO) 8586–1, 1993. *Sensory analysis. General guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors*. Switzerland.
- International Standardization Organization (ISO) 13299, 1998. *Sensory analysis - methodology - general guidance for establishing a sensory profile*, Genève, Switzerland.
- International Standardization Organization (ISO) 15214-08, 1998. *Microbiology of food and animal feeding stuffs - Horizontal method for enumeration of Mesophilic Lactic Acid Bacteria*, Genève, Switzerland.
- International Standardization Organization (ISO) 13720, 2000. *Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Pseudomonas spp.*, Genève, Switzerland.
- Jeyasekaran G., Ganesan P., Anandaraj R., Jeya Shakila R. and Sukumar D. 2006. Quantitative and qualitative studies on the bacteriological quality of Indian white shrimp (*Penaeus indicus*) stored in dry ice. *Food Microbiology*, 23: 526–533. doi:10.1016/j.fm.2005.09.009
- Lalitha K.V., and Surendran P.K. 2006. Microbiological changes in farm reared freshwater prawns (*Macrobrachium rosenbergii* de Man) in ice. *Food Control*, 17: 802-807. doi:10.1016/j.foodcont.2005.05.005
- Liu, H., Du, Y., Wang, X. and Sun, L. 2004. Chitosan kills bacteria through cell membrane damage. *International Journal of Food Microbiology*, 95: 147–155. doi:10.1016/j.ijfoodmicro.2004.01.022
- Liu, X., Yun, L., Dong, Z., Zhi, L. and Kang, D. 2001. Antibacterial action of chitosan and carboxymethylated chitosan. *Journal of Applied Polymer Science*, 79(7): 1324-1335. doi: 10.1002/1097-4628(20010214)79:7<1324:AID-APP210>3.0.CO;2-L
- Lopez-Caballero, M. E., Martinez-Alvarez, O., Gomez-Guillen, M. C. and Montero, P. 2007. Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formulations during chilled storage. *International Journal of Food Science and Technology*, 42: 1029–1038. doi: 10.1111/j.1365-2621.2006.01328.x
- Malle, P. and Poumeyrol, M. 1989. A new chemical criterium for the quality control of fish: Trimethylamine /Total Volatile Basic Nitrogen (%). *Journal of Food Protection*, 52: 419-423.
- Manthey, M. and Oehlenschlaeger, J. 1983. Sensorische Bewertung der filets antarktischeifischebeitiefgefrierlagerung lebensmittel. *Lebensmittel-Wissenschaft und Technologie*, 16: 172-175.
- McBride, N. T. M., Hogan, S. A. and Kerry, J. P. 2007. Comparative addition of rosemary extract and additives on sensory properties of retail packaged beef. *International Journal of Food Science and Technology*, 42: 1201–1207. doi: 10.1111/j.1365-2621.2006.01342.x
- Mendes, R., Huidobro, A. and Lopez-Caballero, M. E. 2002. Indole levels in deepwater pink shrimp (*Parapenaeus longirostris*) from Portuguese coast effect of temperature abuse. *European Food Research and Technology*, 214: 125–130. doi: 10.1007/s00217-001-0419-4
- Mendes, R. 2006. *Guidebook on melanosis inhibitors and processing technology of crustaceans*. INIAP/IPIMAR: Project QLK1-CT-2002-71517 (CRUSTAMEL New approaches to the crustaceans prevention of melanosis and quality indices), p.41.
- Mohan, C. O., Ravishankar, C. N., Lalitha, K. V. and Srinivasa Gopal, T. K. 2012. Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (*Sardinella longiceps*) during chilled storage. *Food Hydrocolloid*, 26(1): 167-174. doi:10.1016/j.foodhyd.2011.05.005
- Muşabak, C. 2008. *Effect of chitosan coating and modified atmosphere packaging on chemical properties of Atlantic bonito (Sardasarda) fillets*. MSc Thesis. Atatürk University, Graduate School of Natural and Applied Sciences, Erzurum, Turkey.
- Niamnuy, C., Devahastin, S. and Soponronnarit, S. 2007. Quality changes of shrimp during boiling in salt solution. *Journal of Food Science*, 72: 289–297. doi: 10.1111/j.1750-3841.2007.00349.x
- Nirmal, N. P. and Benjakul, S. 2009. Effect of ferulic acid on inhibition of polyphenoloxidase and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. *Food Chemistry*, 116: 323–331. doi:10.1016/j.foodchem.2009.02.054
- Nirmal, N. P. and Benjakul, S. 2010. Effect of catechin and ferulic acid on melanosis and quality of Pacific white shrimp subjected to prior freeze–thawing during refrigerated storage. *Food Control*, 21: 1263–1271. doi:10.1016/j.foodcont.2010.02.015
- Nirmal, N.P. and Benjakul, S. 2012. Effect of green tea extract in combination with ascorbic acid on the retardation of melanosis and quality changes of pacific

- white shrimp during iced storage. Food Bioprocess Technology, 5: 2941–2951. doi:10.1007/s11947-010-0483-5
- Pardio, V. T., Waliszewski, K. N. and Zuniga, P. 2011. Biochemical, microbiological and sensory changes in shrimp (*Panaeus aztecus*) dipped in different solutions using face-centred central composite design. International Journal of Food Science and Technology, 46: 305–314. doi: 10.1111/j.1365-2621.2010.02474.x
- Ray, B. 2004. Fundamental food microbiology, 3 ed., CRC Press, Boca Raton, FL.
- Rinaudo, M. 2006. Chitin and chitosan: Properties and applications. Progress in Polymer Science, 31: 603-632. doi:10.1016/j.progpolymsci.2006.06.001
- Shahidi, F., Arachchi, J. K. V. and Jeon, Y. J. 1999. Food applications of chitin and chitosans. Trends in Food Science and Technology, 10: 37-51. doi:10.1016/S0924-2244(99)00017-5
- Shamshad, S. I., Kher-un-Nisa, R. M., Zuberi, R. and Qadri, R. B. 1990. Shelf life of shrimp (*Penaeus merguensis*) stored at different temperatures. Journal of Food Science, 55: 1201-1206. doi: 10.1111/j.1365-2621.1990.tb03898.x
- SPSS, 2001. Statistical Package for windows. Ver. 11.0, SPSS, Inc., Chicago.
- Stockemer, J. and Nieper, L. 1984. Parameter zur Beurteilung der Verderbs von Nordsee- Krabben (*Crangon crangon*). Archive Lebensmittelhygiene, 35: 1-24.
- Terbojevich, M. and Muzarelli, R. A. A. 2000. Chitosan. In: Handbook of hydrocolloids, Eds: Philips, G. O., Williams, P. A., Woodhead Publishing Ltd. Press, Cambridge, UK.
- Theron, M. M. and Lues, J. F. R. 2010. Organic acids and food preservation. CRC Press, Boca Raton.
- Tsai, G. J., Su, W. H., Chen, H. C. and Pan, C. L. 2002. Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. Fisheries Science, 68(1): 170-177. doi: 10.1046/j.1444-2906.2002.00404.x
- Uçak, I., Özogul, Y. and Durmuş, M. 2011. The effects of rosemary extract combination with vacuum packing on the quality changes of Atlantic mackerel fish burgers. International Journal of Food Science and Technology, 46(6): 1157–1163. doi: 10.1111/j.1365-2621.2011.02610.x
- Varlık, C., Baygar, T., Özden, Ö., Erkan, N. and Metin, S. 2000. Sensory evaluation and determination of some physical and chemical characteristics of shrimp (*Parapenaeus longirostris*, LUCAS 1846) during cold storage. Turkish Journal of Veterinary and Animal Sciences, 24: 181-185.
- Varlık, C., Bostan, K. and Bingol, E. B. 2014. Effects of treatment with chitosan and antimelanogenesis agents on discoloration of chilled and frozen stored shrimp. Medycyna Weterynaryjna, 70(6): 357-361.
- Xu, Y. S., Kim, K. M., Hanna, M. A. and Nag, D. 2005. Chitosan-starch composite film: preparation and characterization. Industrial Crops and Products, 21: 185-192. doi:10.1016/j.indcrop.2004.03.002
- Yingyuad, S., Ruamsin, S., Reekprkhon, D., Douglas, S., Pongamphai, S. and Siripatrawan, U. 2006. Effect of chitosan coating and vacuum packaging on the quality of refrigerated grilled pork. Packaging Technology and Science, 19: 149–157. doi: 10.1002/pts.717